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Review

Higher-throughput, label-free, real-time molecular interaction analysis

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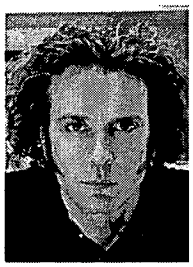
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Molecular interaction analysis involves characterizing how molecular complexes form and break down over time. This dynamic information about biological processes at the molecular level has become an essential component of drug discovery and life science research. Interest in, and application of, label-free interaction technology has grown considerably over the past 10 years. Label-free assays can be faster to set up than traditional methods such as fluorescent or radioligand assays, and they also minimize the worry that the label itself may alter the binding interaction. Although label-free biosensor technology is a well-established analytical tool for kinetic, affinity, and concentration analyses, its application in a high-throughput setting has been limited. This is about to change as a number of instrument manufacturers have released or are developing next-generation biosensors geared at increasing sample throughput.

The basics

In an optical biosensor experiment, a population of one binding partner is tethered to a sensor surface and an aliquot of the other partner is injected across this surface (Fig. 1A). Fig. 1B shows a schematic of the main features of a biosensor binding profile, often referred to as a sensorgram. During the association phase, analyte is exposed to the sensor surface. The optical detection system measures the change in refractive index of the buffer near the sensor surface as analyte mass accumulates on the surface. If the reaction is allowed to proceed long enough, equilibrium may be achieved. A key advantage of biosensor technology is that the amount of complex formed is measured in the presence of free material, unlike filter-binding or pull-down assays that require washing steps before quantitation. This avoids perturbing the reaction equilibrium and makes it possible to characterize very weak or transient interactions. During the dissociation phase, the surface is washed with buffer and the signal is monitored as the complexes break down over time. By tracking binding events in real time, it is possible to derive kinetic information about the interaction mechanism. Also, by changing analysis parameters (e.g., buffer, temperature, pH, analyte concentration) and testing a family of analytes (e.g., related compounds or, protein mutants), we can characterize an interaction in detail. These features make optical biosensor technology a popular biophysical tool.

Although there has been an exponential increase in the number and range of available biosensors, we focus this technology update primarily on higher throughput systems. Even though the instruments we describe may employ different optical systems, we do not describe here the details on how each detection system works. The reason for this is rather simple: one does not need to under-

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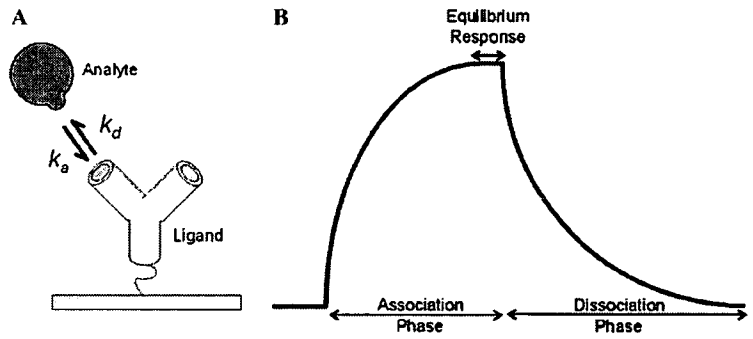

Fig. 1. Elements of an optical biosensor analysis. (A) Assay design. Analyte in solution binds to a target ligand immobilized on the sensor surface. (B) Biosensor output. The response increases during the association phase as analyte flows across, and binds to, the immobilized ligand. A plateau in the response at the end of the association phase indicates that the system has reached equilibrium. The response decreases during the dissociation phase as the surface is washed with buffer and the analyte dissociates from the surface.

stand the principles behind any of the detectors to implement the technology correctly. For our purposes, it is sufficient to know that all of these biosensors measure changes in refractive index of the solution very near the sensor surface. If one is interested in the underlying optical phenomena of each instrument, we suggest visiting the manufacturers' websites (listed in Table 1). Instead, we focus our attention on the differences and similarities in how samples are delivered and processed by the various technologies since sample delivery correlates directly with throughput and throughput is the most critical unmet need of label-free interaction analysis.

Instrument manufacturers are employing a variety of formats to improve throughput—one analyte against many targets, many analytes against one target, and many analytes against many targets. Here we subdivide the instruments we review by these different analysis formats. To appreciate today's biosensor technology, we briefly review the evolution of the technology with an eye on sample delivery.

One-on-one approach

In 1990, Pharmacia Biosensor released the first commercially viable optical biosensor platform called Biacore. This platform was truly revolutionary in its assemblage of microfluidic, optic, chemical, and robotic technologies. This first-edition instrument had four flow cells, but they could only be addressed separately (Fig. 2A): one analyte could be studied over one target at a time. Typically, the same sample would be tested over the target surface and then over a reference surface in a separate injection. Although this was a bit tedious, the instrument was fully automated and capable of overnight unattended operation. This technology may seem primitive compared with later-generation platforms, but it is important to recognize that this technology launched the field of label-free real-time molecular interaction analysis. Over the past 16 years, optical biosensors have evolved from a niche instrument directed toward examining a handful of straightforward macromolecular interactions to a widely accepted technology suitable for studying biological systems ranging from small molecules, carbohydrates, oligonucleotides, receptors, and antibodies to lipids, viruses, and whole cells. A good gauge of the success of biosensor technology is that more than 1000 publications each year include data collected from commercial biosensors [1,2].

One-on-several approach

Whereas the first label-free real-time biosensor revolutionized molecular interaction analysis, the next breakthrough made it impossible to turn back. Around 1996, Pharmacia spun the biosensor division out as an indepen-

Table 1
Array biosensor platforms

Manufacturer	Website	Technology[a]
Biacore	www.biacore.com	Biacore, 2000, 3000, T100, Flexchip, S51, and A100
Axela Biosensors	www.axelabiosensors.com	dotLab
Toyobo	www.toyobo.co.jp/bio	MultiSPRinter
Lumera	www.lumera.com	Proteomic Processor
GenOptics	www.genoptics-spr.com	SPRi-Plex
SRU Biosystems	www.srubiosystems.com	BIND
Corning	www.corning.com	Epic
ForteBIO	www.fortebio.com	Octet
Bio-Rad	www.biorad.com	ProteOn XPR36

[a] Technology (or technologies) discussed in this review (not necessarily all platforms offered by a manufacturer).

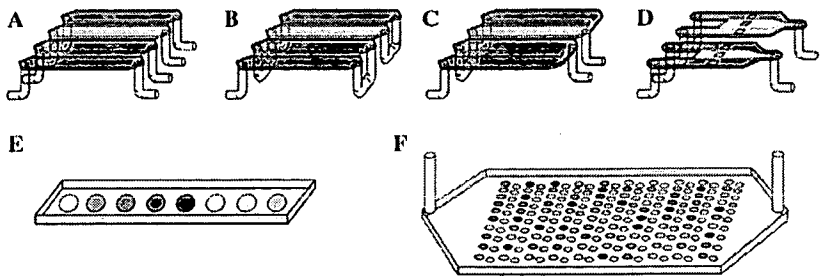

Fig. 2. Schematic of flow cell configurations in the original Biacore instrument (A), the Biacore 2000 and 3000 (B), the Biacore T100 (C), the Biacore S51 (D), the Axela Biosensors dotLab (E), and the Biacore Flexchip (F). In panels D–F, the reference surfaces are shown in yellow. (For interpretation of the reference to color in this figure legend, the reader is referred to the Web version of this article.)

dent company called Biacore AB and released a new platform called Biacore 2000. Although it was similar to the original biosensor in its core features, the major advance in the technology was a microfluidic design that allowed the user to run one analyte sample over four flow cell surfaces independently or in series (Fig. 2B). This made it possible to automatically immobilize separate target ligands and then collect data from a reference surface and three reaction surfaces at the same time. The result was increased throughput and significantly improved data quality, making it possible to detect low-molecular-weight analytes (~200 Da). A few years later, an updated system called Biacore 3000 was released with a similar flow cell design but having a detector with an expanded dynamic range that made it possible to monitor interactions in the presence of highly refractive index buffers such as crude sera and dimethyl sulfoxide (DMSO)[1]. Both instruments are capable of processing samples from two standard 96-well plates. Together, the Biacore 2000 and 3000 series currently are the most used biosensors in the field.

Biacore AB continues to make improvements to its four-flow cell configuration sensor technology. One of Biacore AB's most recently released platforms is called the T100. In this instrument, two pairs of flow cells are connected within the same plane and form "U-shaped" flow paths (Fig. 2C) [3]. By using a combination of three ports in this flow cell, it is possible to immobilize a target on one arm of the "U" and use the unmodified surface of the other arm as a reference. This redesign shortens the distance between the target and reference surface, thereby improving referencing. In contrast, the flow cells in Biacore 2000 and 3000 instruments are joined through more complex fluidics between and under the flow cells (Fig. 2B). The use of the U flow cell improves data quality while retaining many of the robust features of the previous four-channel flow cell designs.

Realizing that referencing was an important way of improving data quality, Biacore AB developed a novel approach to flow cell design in its S51 systems, a platform that was engineered specifically to support small molecule drug discovery and development. The S51 uses "Y-shaped" flow cells (Fig. 2D) and the technique of hydrodynamic addressing to immobilize target molecules along the outer edges of the flow cell while maintaining an unmodified region in the center [4]. Small detector windows are positioned in the middle of the flow path within each region. The center region serves as an internal reference, making it possible to collect high-resolution data for very small analytes. This increase in sensitivity also makes it possible to collect binding data on targets with low activity. The S51 technology has been implemented by a number of pharmaceutical companies as a secondary screening tool. The Biacore T100 and S51 instruments both can accommodate samples from one 96- or 384-well plate while processing one sample at a time.

One-on-many approach

Given the positive impact that Biacore's technology has had on interaction analysis, it is not surprising to see other manufacturers developing systems for the label-free market. One approach to improving throughput is to increase the number of targets examined at a time—the one-on-many approach. For example, Axela Biosensors' dotLab system involves producing a linear array of eight target spots within a flow channel (Fig. 2E). A single analyte is tested simultaneously for binding to all of the target spots. Axela Biosensors is working on producing addressable arrays by using a variety of capturing surfaces within the channel to immobilize a variety of tagged ligands at one time. These disposable cartridges could be particularly useful in diagnostic applications.

Following the adage that more is more, several manufacturers are significantly expanding the number of targets into two-dimensional arrays. In this approach, one analyte is flowed across targets that have been spotted in a large-format flow cell (Fig. 2F). Using imaging technology, binding data can be collected from both the target spots and unmodified regions between the spots that are used for internal referencing. Typical applications for these instruments include monoclonal antibody screening and characterization of peptide and oligonucleotide arrays.

Biacore's Flexchip system was the first commercially available high-density array platform and is capable of analyzing one analyte against 400 target spots at one time.

[1] *Abbreviations used:* DMSO, dimethyl sulfoxide; cDNA, complementary DNA; ELISA, enzyme-linked immunosorbent assay.

Recently, researchers have begun to demonstrate Flexchip's utility in higher-throughput biosensor-based screening applications, including epitope mapping [5], concentration analyses [6], and protein expression profiling [7]. As the first step toward fully recognizing this platform's potential for monitoring interactions in real time, researchers have reported Flexchip-obtained kinetic and affinity parameters for various antibody systems [8,9].

Toyobo's MultiSPRinter is an array-based sensor platform distributed in Japan. This system is interesting because it provides the customer with a complete package, including a spotter and detection system. Exemplifying the flexibility of this biosensor, researchers have used this platform to monitor DNA hybridization [10], track protein expression [11], and obtain kinetics for protein binding to an array of related double-stranded DNAs [12].

The pin-spotting methods commonly used to array targets work well for immobilizing peptides, oligonucleotides, and stable proteins such as antibodies. However, because spotting uses small transfer volumes and requires drying the target on the chip, this method might not be universally applicable to preparing all protein arrays. Many proteins become denatured when dried onto surfaces, are not available at high enough concentrations, and/or often are in crude preparations. To tackle this problem, researchers at GenOptics have developed novel in-solution target immobilization chemistries to complement their SPRi-Plex biosensor: with electrocopolymerization, pyrrole-conjugated targets are covalently coupled to pyrrole-modified spots on the sensor surface. Using this system, which tracks binding at up to 300 target spots, investigators have characterized DNA, peptide, and antibody interactions [13–16].

Lumera is taking the quest to produce protein arrays to the next level. This optics company is in the final phases of testing its array-based system, called the Proteomic Processor, which is capable of examining more than 1000 target spots at one time [17]. Lumera's future plans include launching an on-chip in vitro translation system in which complementary DNA (cDNA) will be spotted on the sensor surface to translate protein with a coil fusion tag that interacts with a coil peptide copartner prepatterned on the chip surface (NanoCapture microarrays). This translation system has the potential to eliminate laborious protein production and purification steps. There are, of course, challenges in the routine application of in vitro translation, but this approach could provide a simple method of generating content for protein arrays.

Plate-based systems

To move biosensor technology to the fast lane of drug discovery, it is apparent that the number of analytes that can be tested needs to increase significantly. Two companies, Corning and SRU Biosystems, have developed similar approaches to increasing sample throughput in their Epic [18–20] and BIND [21,22] systems, respectively. In these technologies, the sensor surface is incorporated directly into the bottom of specially modified, but standard-sized, 96- or 384-well plates. Targets are loaded into these plates and can be tested simultaneously against many analytes. These plate systems meld well with existing plate-based high-throughput robotics that are common in pharmaceutical companies for efficient sample delivery and sample tracking. Although binding data can be collected in a real-time mode, this feature is used primarily for assay development. For high-throughput operation, data output is primarily end-point measurements, so it is better to think of these systems as label-free enzyme-linked immunosorbent assays (ELISAs). These types of systems could be good for handling assays for targets that are difficult to prepare in an ELISA format or for compounds that interfere with fluorescent-based systems.

ForteBIO, with its Octet technology, has taken a different approach to testing plates of samples. In this system, sensors are incorporated into disposable detector tips, which are coated with target and dipped into wells containing analyte solutions. In this configuration, the binding of eight analyte–target pairs can be tracked at one time. After each set of analyses, the used tips are discarded and a fresh set of tips is used to examine the next eight analyte solutions. The system uses standard well plates and the analyte solutions can be fully recovered. Another advantage of each of these plate-based technologies is that they can be used to test binding in very crude material because clogging is not an issue, as it can be with microfluidic flow-through systems.

High-resolution parallel processing

The final set of instruments we highlight involves novel developments of flow-through systems. Biacore's A100 and Bio-Rad's ProteOn technologies can perform parallel sample analyses while maintaining high data quality.

Biacore's A100 is a 4×4 parallel array processing instrument that represents an expansion of the S51 technology. The A100 has four independent flow systems that transfer analytes from plates (96- or 384-well) to four independent hydrodynamic addressing flow cells like those shown in Fig. 2D. In each A100 flow cell, however, four targets can be immobilized and an unmodified spot in the center serves as the reference. The system can be used for applications ranging from antibody screening to small molecule analysis [23,24].

Bio-Rad's ProteOn XPR36 instrument incorporates a unique approach to generating a two-dimensional array. The system contains six parallel flow channels that can be used to uniformly immobilize strips of six targets on the sensor surface (Fig. 3A). The fluidic system can automatically rotate 90° to create a 36-target array (Fig. 3B). Reference spots are automatically formed before and after each reaction spot to permit internal referencing. The ProteOn system also introduces a novel concept in kinetic analysis referred to as "one-shot" kinetics. With this approach, one can simultaneously test six different concentrations of

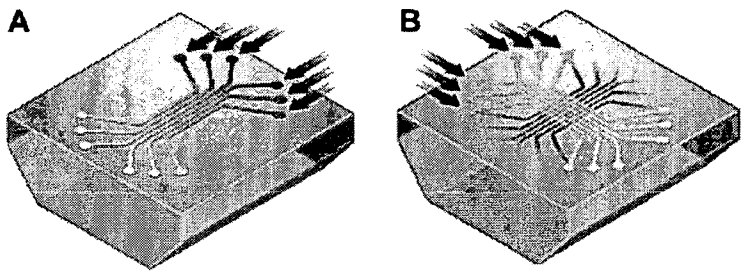

Fig. 3. Flow cell configuration for Bio-Rad ProteOn XPR36. (A) Ligand targets are immobilized in six parallel flow channels. (B) After the chip is rotated 90°, six analytes are injected perpendicular to the channels of immobilized targets. Interstitial spaces in each channel are used for referencing.

analyte over the different target surfaces, thereby generating a full analyte concentration series with one injection [25,26]. This reduces analysis time significantly and eliminates the need for surface regeneration. Alternatively, the six channels can be used to screen a single concentration of six compounds against six different target proteins with each injection or any combination in between. The ProteOn XPR36 currently accommodates samples from two 96-well plates.

Summary

The burgeoning number of commercial biosensors geared toward higher throughput analysis clearly demonstrates that the market for this technology is expanding. Beyond throughput, the second most important issue is sensitivity. At this time, we have tried to steer clear of comparing systems based on sensitivity, principally because it is difficult to do so without a benchmark study using the same molecular system to evaluate every instrument. Suffice it to say that there is likely to be a trade-off between resolution and throughput. For example, the sensitivity of the two-dimensional array-based systems that we discussed likely is not high enough at this stage to allow direct detection of the binding of small molecules to immobilized targets. So, in many ways, more could in fact mean less.

It is, however, important to remember that the primary factor that determines the quality of any biosensor data set is the quality of the target itself and how it is immobilized. None of these sensors can yet take a denatured and precipitated protein and turn it into a fully active target on the sensor surface. (If there was such a sensor, we would have highlighted it.) In contrast, low-percentage activity often is not a hindrance for enzymatic-, radioligand-, and fluorescent-based assays. Because these assays can be 1000 times more sensitive than optical biosensors, they often are used to analyze targets that are not especially pure or highly active (whether this is actually a good idea is debatable).

It is also important to recognize that most optical biosensor assays are performed as direct binding assays. For small molecule work, for instance, we can use the sensor to determine whether a compound actually interacts with a particular target. These direct binding assays, on their own, do not tell us whether the compound actually inhibits or activates a particular target. For this reason, most pharmaceutical companies will continue to use activity-based assays as a primary screening tool. In this setting, biosensors are ideal tools to be used for secondary screening to confirm whether the hit is real and, if so, to provide dynamic information about its interaction with the target. Information on how fast something binds and the half-life of the complex is becoming an essential part of the optimization process for both small molecules and biopharmaceuticals.

We applaud these biosensor manufacturers for developing tools that will increase the speed of interaction research, ultimately leading to a better understanding of both basic biology and improved health care.

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